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Neurobiology: Fly Gyro-Vision

Flies stay on course using a combination of high performance vision and a specialized sensory gyroscope. A new study reveals that these disparate modalities are wired together.

Mark A. Frye

So you think you can see? Human retinal ganglion cells are roughly predicted to transmit visual information at the equivalent of 10 bits per second [1], which is likely an underestimate of photoreceptor capacity. Foveal photoreceptors may transmit up to 100 bits per second in full daylight, but this performance nevertheless pales in comparison to the 1000 bits per second transmitted by the photoreceptors of a flesh fly [2]. Put another way, the 16 Hz flicker fusion cut-off for human cones in part enables us to be fooled into perceiving smooth motion in movies displayed at 30 frames per second. Yet flies perceive image frequencies well in excess of 100 Hz, and would therefore see our movie as something akin to a slide show.

Though fly visual transduction is the fastest yet measured in any animal, the extreme retinal image speeds achieved during routine flight maneuvers [3] are well beyond those which can be effectively compensated by visuo-motor reflexes [4]. Therefore, like humans, flies reduce the corrupting influence of image blur during locomotion by actively moving their heads to stabilize their gaze [5]. Just like a ballet dancer in a pirouette fixes his gaze on one spot to maintain stability, a fly steering its body into a turn contra-rotates its head to keep the visual world reasonably still [6]. A new study [7] shows that the extreme visual capabilities of flies are due in part to the convergence of multiple sensory modalities upon the control of head posture for stable gaze.

Maintaining stable gaze while chasing down a visual target, such as a territorial invader or potential mate, requires adjusting head posture to fixate the visual world and also to counteract movements of the body. Visual inputs from the compound eyes are segregated into parallel processing pathways specialized to encode patterns of panoramic optic flow generated during self motion [8,9], or small moving targets generated by prey or conspecifics [10]. Body dynamics are encoded by gyroscopic sensory organs called halteres that beat back and forth like the wings and during body rotation generate out of plane reaction forces that are detected by mechanoreceptors at their base [11].

Visual and mechanosensory signals converge on the neck musculoskeletal system to pivot the head [7]. Thus, if a visual target drifts laterally (Figure 1A), visual activation of the neck motor system produces a compensatory head turn (Figure 1B). Similarly, mechanical deflection of the body and haltere sensors by a gust of wind evokes a contra-rotating compensatory head movement (Figure 1C). It would thus appear that the visual and mechanosensory systems are well synchronized for the task of stabilizing gaze.

"Ay, there's the rub": haltere sensory neurons respond to stimulation within microseconds [12], and in turn mediate changes in head postural position within three milliseconds of a sensory disturbance [13]. This behavioral latency is ten times shorter than the activation delay within visual motion processing neurons [14]. The time discrepancy is evident within the very earliest stages of sensory transduction. In contrast to the rapid direct activation of ion channels in mechanoreceptors, photoreceptor signaling in flies uses a comparatively sluggish G-protein signaling cascade. Add to that the

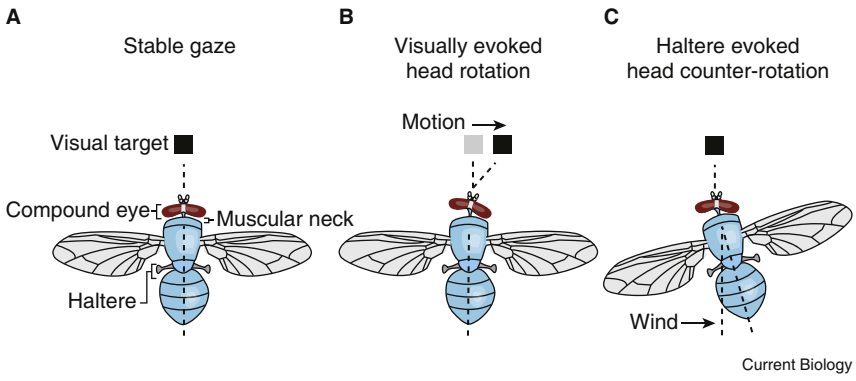

Figure 1. Head movements that stabilize the direction of gaze are evoked by two sensory systems.

(A) A visual target such as another fly is fixated on the forward-looking compound eye. The articulated head is moved by muscles that receive input from the compound eyes and also from gyroscopic sense organs called halteres. (B) Movement of the visual target activates the neck muscles to turn the head and stabilize gaze. (C) A wind gust on the body is detected by the halteres and results in coaxial contra-rotation of the head to stabilize gaze.

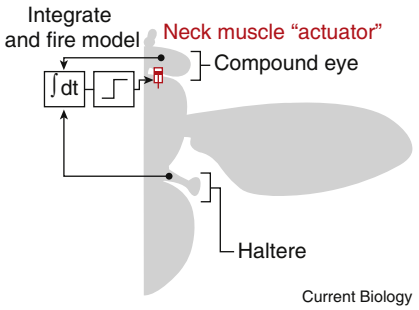

Figure 2. Model for multisensory control of gaze.

Subthreshold visual signals from the compound eye and the mechanosensory signals from the halteres are integrated to bring the neck steering muscle (represented by a hydraulic actuator) to firing threshold. Either modality alone is insufficient to achieve the threshold level needed for actuation.

numerous computations required for vision and in the end, fast as it may be, the fly visual system cannot keep pace with the temporal performance of the haltere sensory system, not by a long shot.

The kinetics of the two sensory systems are reflected to some extent in the frequency tuning of visual and haltere mediated flight reflexes; visual stabilization behaviors peak for slow stimuli, whereas mechanosensory equilibrium behaviors increase monotonically with the rate of stimulation [15]. Therefore, at both cellular and behavioral levels, the output signals from the two sensory pathways operate on very different time scales. How are the two resolved for gaze stabilization?

To address this question, Huston and Krapp [7] devised a method to record the intracellular membrane potential of neck motor neurons while visually stimulating the compound eyes and mechanically stimulating the halteres. The visual stimuli were projected from a standard television-like display. Stimulating the halteres of an immobilized animal required more ingenuity; the solution was to glue a metal particle onto the haltere and oscillate the appendage in a cycling magnetic field. Thus, the neck motor neuron could be excited by visual input, mechanosensory input, or both simultaneously.

Huston and Krapp [7] found that some of the neck motor neurons were excited by visual stimuli alone, thus corroborating previous findings suggesting that this group of muscles is directly excited by optic flow-sensitive interneurons in the brain [16]. However, another group of neck motor neurons showed visual excitation only when co-activated with haltere sensory input. Neither visual nor mechanosensory stimuli alone elicited action potentials. Only when the haltere was being oscillated did concomitant visual stimuli result in robust directionally selective spiking responses.

The general finding that visual and mechanosensory stimuli both activate the neck motor system [7] was somewhat expected because it had been established that each sensory modality evokes head movements (Figure 1). The "eureka" came from the specific way that the different sensory cues were fused. Haltere input excites the neck motor circuit with each haltere beat, but the membrane excitation is below the threshold required to fire an action potential. Visual excitation alone is also subthreshold, but the two converging signals are temporally integrated to bring the motor neuron to firing threshold, activate the muscle, and turn the head (Figure 2).

The specific advantage here is that the relatively slow tonic visual signals are effectively gated by the fast wingbeat-synchronous mechanosensory signals. In this way the neck muscle system is triggered in temporal register with the beating halteres. That is to say the haltere input 'clocks' the visual input. Therefore, one input channel (haltere) effectively transforms the other input channel (compound eye) into a fast behaviorally relevant motor code. The disparate time scales are effectively resolved.

The fusion of inputs from the compound eyes and halteres helps to explain why flies only turn their heads to track visual motion when the halteres are beating - during flight or walking [13,17]. But what is the advantage of this complicated multimodal convergence? The answer lies within the organization of related sensory-motor transformations.

It turns out that haltere sensory signals have the same general influence over wing steering muscles that they have over neck muscles — synchronous excitatory integration toward firing threshold [18]. A gust of wind that twists the body in flight would therefore excite the halteres and evoke a rapid corrective response in wing kinematics. Additionally, it would also seem obvious that in order to respond to visual movement, visual signals should activate the wing muscles. Strangely enough there is no evidence directly linking visual signals to the wing motor system. Instead, visual signals project directly to the haltere muscles in a manner very similar to the neck motor system [19].

We find in flies several multisensory reflex arcs in which visual signals control the halteres, the halteres control the wings, and both visual and haltere signals control the neck to move the eyes in turn modifying the incoming visual signals. It stands to reason that stabilizing the direction of gaze is critically important to the overall flight control effort, because information capacity is irretrievably degraded when the speed of optic flow exceeds the encoding capacity of motion processing pathways.

It therefore seems to be highly advantageous that fast mechanosensory feedback effectively intercedes in the control of head and wing kinematics. When these results are considered alongside an emerging literature on cross-modal visual, olfactory, and antennal mechanosensory mediated behavior in flies [20], the emerging multimodal control systems appear to provide both a mechanistic basis for the extreme sensory-motor performance of flies in particular, and also a general conceptual framework for how high-level computational behavior emerges from low-level circuit interactions and algorithms.

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Metastasis: Alone or Together?

Recent studies of carcinoma progression reveal the distinct routes of dissemination of discrete carcinoma cell populations and suggest that melanoma cell dissemination is linked to differentiation rather than stemness status.

Jean Paul Thiery

Classical models of tumor invasion and metastasis implicate the progressive accumulation of genetic and epigenetic alterations in the generation of locally invasive and metastatic tumors. Although clonal in origin, malignant cells rapidly become heterogeneous and coexist with a variable amount of stroma in the tumor. The original dogma was that a small subset of clones becomes susceptible to progress and acquire a metastatic potential [1]. It was later shown that similar clusters of gene expression profiles can be found at different stages of tumorigenesis, suggesting that the metastatic potential was acquired at an early stage by the whole tumor rather than by a subset of malignant cells [2]. More recent studies have revealed that some malignant cells in the primary tumor activate, in part, a complex signaling program to colonize specific organs and subsequently form macrometastases [3]. None of these studies, however, has thoroughly analyzed cell behavior in a primary tumor mass during its expansion.

New imaging techniques have captured the behavior of endothelial cells *in situ* during tumor angiogenesis [4], as well as the behavior of other stromal and malignant cells [5]. Intravital imaging, using multiphoton microscopy, considerably reduces fluorophor bleaching and the production of oxygen radicals and allows for the visualization of different cell behaviors. At the same time, increasing the optical resolution via second harmonic generation allows for the detection of extracellular matrix (ECM) fibers containing helical proteins, such as collagen. With these techniques, studies have demonstrated that some carcinoma cells have a much higher speed of locomotion *in vivo* than in 2D or 3D *in vitro* motility. Also, continuous monitoring of carcinoma cell migration within the extracellular environment has revealed an amoeboid mode of movement of solitary cells that loosely interact with ECM fibers via focal complexes and do not induce tension in cells: carcinoma cells can therefore reach blood vessels and intravasate [5].

Using a similar intravital imaging approach, new findings from Sahai and colleagues [6] have revealed that rat mammary MTLn3 metastatic adenocarcinoma cells, when transplanted into the fat pad of wild-type mice, migrate either as cell collectives or as solitary cells. The solitary cells, constituting about 5% of the carcinoma, move much more rapidly (150 μm/h) than the compact cell clusters and intravasate into blood vessels, whereas cells in clusters preferentially invade the proximal inguinal lymph nodes where they remain mostly immobile. This transient acquisition of motility was found to be driven by transforming growth factor β (TGFβ) signaling, particularly for the solitary cells, which had undergone an epithelial–mesenchymal transition (EMT) [7]. Interestingly, the TGFβ signaling effector Smad2 was localized to the nucleus in these cells, although this localization was transient because metastatic cells, forming large clusters in lymph nodes and in the lung, have a cytoplasmic localization of Smad2. The transient nature of TGFβ signaling was confirmed with a TGFβ-dependent reporter gene; however, TGFβ signaling was found to be active in some non-migratory cells, suggesting that TGFβ signaling may be necessary but not sufficient to induce motility. *In vitro* studies confirmed that TGFβ can induce EMT in carcinoma cells, whereas epidermal growth factor (EGF), not TGFβ, triggered collective cell migration.

TGFβ target genes involved in the switch from collective to single cell motility were identified, including the small GTPases RhoA and RhoC, which are both important for actomyosin contractility; EMT could only be inhibited when both small GTPases were depleted by siRNA. Furthermore, knockdown of the TGFβ targets MRIP, Farp-1, c-Jun or the EGF receptor also reduced cell scattering. Some TGFβ target genes were implicated in the regulation of adherens junctions, whereas others may be instrumental in the control of individual cell locomotion, such as Nedd9, which promotes actin polymerization, or